

UNITED STATES PATENT AND TRADEMARK OFFICE
(Attorney Docket No. MBHB 07-1047-WO-US)

IN RE APPLICATION OF:)	
David Paul Humphreys, et al.)	
)	Examiner: David Blanchard
Serial No. 10/562,807)	
)	
Filed: July 6, 2006)	Group Art Unit: 1643
)	
Title: Modified Antibody Fragments)	Confirmation No.: 6569

DECLARATION OF DAVID P. HUMPHREYS

I, David P. Humphreys, in support of the above-identified patent application, do aver and state as follows:

1. I am a co-inventor of this patent application. I also am the lead author of the scholarly journal article appearing at J. Immun. Methods, 209:193-202 (1997) which has been cited as a prior art reference against this application.
2. I received a First Class honours degree in Molecular Biology and Biochemistry from the University of Durham (UK) in 1992. I received a PhD. from the University of Birmingham (UK) in 1995. My thesis was entitled "Expression of Human Protein Disulphide Isomerase (PDI) in *E. coli*, and protein folding *in vivo*;" this work resulted in two publications in peer reviewed journals. My PhD. research work was sponsored by the predecessor-in-interest of the assignee herein, and I have continued to work for the assignee since that time. For the last eighteen years, I have investigated issues relating to cysteine engineering and disulfide bond architecture in proteins of industrial interest, advancing from post-doctoral

researcher to my present position of Director, Antibody Biology. A list of my published papers, patent applications, and presentations is attached as Exhibit A hereto.

3. At the time of the present invention, it was known that PEGylation of antibodies and antibody fragments could improve serum circulation half-life. The present application arose from our search for a more efficient way of providing an effector molecule such as PEG at the hinge portion of a Fab' fragment. It was known that in order to attach a PEG molecule to the hinge, the cysteine at the hinge would have to be reduced to a thiol by subjecting the entire molecule to a reduction step. At the time of the invention, it was common wisdom in the art that the interchain disulfide bond between the heavy and light chains had to remain intact during this reduction step in order to preserve the antigen-binding affinity of the Fab' fragment. It was believed that if the interchain bond were absent, then the light chain would disassociate from the heavy chain *in vivo*, particularly once the molecule was PEGylated. Therefore, reduction reactions on the Fab' fragment to reduce the hinge cysteine were carried out under mild conditions, so that only the hinge cysteine and not the interchain bond cysteines would be reduced. These mild reactions conditions resulted in inefficient PEGylation reactions.
4. Prior to the present invention, mutations to create Fab' antibody fragments lacking inter-chain (LC-HC) bonds were known, but these Fab' fragments had not been PEGylated. Specifically, Rodrigues et al (1993) and Humphreys et al (1997) (of which I am the principle author) taught that Fab' lacking inter-chain disulphide bonds could be expressed and purified using normal methods. Both of these references are directed to improving the efficiency of F(ab')₂ formation *in vitro* from Fab' fragments. Neither Rodrigues et al. (1993) nor Humphreys et al. (1997) taught or suggested PEGylation of those Fab' fragments. None of these works, or to my

knowledge any other prior art, taught or suggested that Fab' lacking inter-chain disulphide bonds might be suitable for *in vivo* applications.

5. Other references from that time period demonstrate the then-prevailing belief in the importance of maintaining the interchain disulphide bond. At the time of our work a wide spectrum of diseases associated with cancers of the blood were known to involve the production and deposition on and in organs of patients of 'abnormal immunoglobulins.' These include amongst others 'heavy chain deposition disease' and 'light chain deposition disease.' One of the well known manifestations of such diseases are the presence of 'Bence-Jones' proteins, which are light chain dimers that can be detected in the urine (Bradwell et al., The Lancet (2003) 361: 489-491). Bence Jones proteins appear to have multiple origins including over-expression of LC, LC variable region mutations and truncations in antibody heavy chain genes, typically C_H1, C_H1-hinge or even C_H1-hinge-C_H2 (Cogne et al, J. American Soc. Hematology (1992) 79:2181-2195). In one specific example, the human myeloma IgG1 'Dob' was shown to have a 15 amino acid deletion of the hinge sequence EPKSCDKTHTCPPCP (Steiner and Lopes Biochemistry (1979) 18:4054-4067). Hence 'Dob' was known to lack the C_H1 cysteine involved in the LC-HC disulphide bond and was shown to form an aberrant LC-LC disulphide bond. In light of this combined knowledge, we were concerned that Fab-PEG or Fab'-PEG with long serum half-life, lacking an interchain LC-HC disulphide might be capable of LC loss or LC exchange.
6. Humphreys et al., (1998), of which I am the principle author, showed that increased stability of inter-hinge linkages through increased numbers of disulphide bonds resulted in increased serum permanence of F(ab')₂ molecules in rats. The fragments in this study had intact interchain disulfide bonds, consistent with the prevailing belief that the natural inter-chain (LC-

HC) disulphide might indeed be required for full stability of molecules with longer circulation times, thus teaching one skilled in the art away from the use of fragments having no inter-chain covalent bonds. To the contrary, Hong and Nisonoff (1965) reported that breakage of inter-chain (LC-HC) bonds resulted in at least some dissociation of light chain from heavy chain of a rabbit antibody, as observed during chromatographic purification. This suggested that the absence of an inter-chain covalent bond presented at least some risk of LC loss or exchange in circulating serum. Hence at the time of the present invention we were uncertain as to how robust Fab'-PEG lacking inter-chain (LC-HC) bonds would be during circulation in serum over 1-2 weeks.

7. Fab' fragments that had been site-specifically mono-PEGylated at the hinge were known, but only those with intact inter-chain (LC-HC) bonds (cf. Chapman et al. (1999)). This is consistent with the general understanding in the art at that time that the inter-chain bond had to be intact in order to maintain good binding affinity to antigen.
8. Fab' molecules lacking inter-chain (LC-HC) bonds for PEGylation were conceived by me and the co-inventors herein. At the time of the present invention, little was known about how to predict the serum circulation half life of antibody and Fab' molecules with altered structures. It was known that 'unstable' molecules such as scFv and human IgG4 were prone to 'domain exchange' both *in vitro* and *in vivo*. Since the long serum permanence was conveyed by the PEG molecule which was covalently attached to the heavy chain, it seemed highly plausible that in Fab'-PEG lacking inter-chain (LC-HC) bonds the light chain might be exchanged or lost in the circulation. This would have resulted in loss of antigen binding function (loss of efficacy) or increased clearance of the protein through precipitation, aggregation or proteolysis.

9. The middle hinge of human IgG4 differs from that of human IgG1 in both sequence (CPSC vs CPPC) and in ability to form a ‘half-molecule.’ It was known at the time that IgG4 was able to ‘domain exchange’ in plasma to form monovalent, bispecific molecules, Schuurman et al., 1999. It had been proposed that this domain exchange was enabled by the ‘unstable’ IgG4 hinge sequence (Aalberse et al. 1999), mutation of which to CPPC was known to effectively stop the formation of the closely linked ‘half-molecule’ *in vitro* (Angal et al., 1993). Hence at the time of our experiment it was known that ineffective interchain disulphide stabilisation could result in efficient exchange between non-covalent protein:protein interfaces in Fc domains encoded by CH2-CH3. It seemed highly plausible that Fab’ light chain could be lost from our molecule in circulating serum. The residues and energetics involved in the homo-interaction of CH3 domain of human IgG1 had been studied and measured.
10. In our experiments, we modified both the light chain and the heavy chain of the Fab’ fragments by replacing the cysteines that had formed the inter-chain disulfide bonds with other amino acids, thereby destroying the interchain bond, and ensuring that the two sites of the interchain bond could not subsequently become sites for PEGylation. As the interchain cysteines were no longer present, we could use stronger reaction conditions during the reduction of the hinge cysteine to the thiol, allowing for more efficient PEGylation at that site. Surprisingly, our modified Fab’-PEG molecules having no covalent bond between the light and heavy chains were active and stable in the circulation of mice for up to 7 days. Further, there was no loss of antigen binding affinity. This result was wholly unexpected, because the literature at the time suggested that the absence of an interchain bond would result in instability of the fragment in terms of loss or exchange of the light chain *in vivo*. We had believed that this instability could be even greater if a large PEG effector molecule was bound to the hinge.

11. I have read the references cited against the present application. Chapman et al. (Nature Biotechnology, 17:780-783, 1999), as discussed above, discloses that Fab' fragments having intact inter-chain disulfide bonds and having a single PEG attached at the hinge region have improved half-lives without loss of antigen-binding affinity. Fig. 2 of the Chapman et al. disclosure specifically shows an intact covalent bond between the light and heavy chains, thus teaching away from the presently claimed invention. To me as one skilled in the art, this reference suggests that in order to have improved stability *in vivo*, an interchain bond is necessary. Chapman does not teach or suggest to one skilled in the art that any modification should be made to either the light chain or the heavy chain of the Fab' fragment.
12. My own paper Humphreys et al. (J. Immun. Methods, 209:193-202, 1997) is directed to the formation of dimeric Fab's (i.e., F(ab')₂) in E. coli., because in clinical applications it is often desirable to have the increased effective binding affinity afforded by a dimeric Fab' (Humphreys et al. at p. 193, second column, lines 5-7). We also demonstrated that Fab' lacking inter-chain disulphide bonds could be expressed and purified using normal methods. We found that Fab' fragments with unmodified $\gamma 1$ hinges gave the greatest F(ab')₂ yield *in vivo* (p. 198, second column, lines 41-43), which would suggest to one skilled in the art that unmodified hinge regions were preferred. This paper is not directed to PEGylation of antibody Fab' fragments, nor does it discuss effector molecules of any kind.
13. In my opinion as one skilled in the art of antibodies, one skilled in the art would not combine these two references in the manner suggested by the patent examiner. Chapman taught that it was desirable for PEGylation to be accomplished with the interchain bond intact, and Humphreys 1997 taught nothing about PEGylation. These two endeavors have different goals. Nor would Humphreys et al.'s disclosure of modified hinge regions and the lack of a light-chain-heavy chain bond have suggested anything to

the skilled artisan about the effects thereof on PEGylation. Nor would one skilled in the art have preferentially selected these aspects of the Humphreys et al. disclosure rather than any of the other parameters studied in the disclosure, i.e., effect of hinge size, Fab' expression levels, tailpiece sequences, and growth conditions. There is no reason for one skilled in the art to combine these references. Moreover, the prevailing view in the art that destroying the interchain bond would diminish antigen binding affinity would lead one skilled in the art away from removal of both interchain cysteines.

14. I note that the Examiner states that in my 1997 article we removed the inter LC-HC disulphide bond by changing the interchain cysteines to serines, in order to minimize incorrect interchain disulphide bonds between hinge regions and other cysteines. In particular, we wanted to minimize such incorrect bonds when the two Fab' fragments come together to form the di(Fab')₂ dimer. The issue of such incorrect bonds does not arise when the fragments are to be used in the form of un-dimerized Fab'.
15. The Singh et al. reference (Analytical Biochemistry, 304(2): 147-156, May 15, 2002) teaches a method of labeling whole antibodies, with non-selective selenol-catalyzed reduction employing labels such as biotin-PEO-maleimide complex having a formula weight of 525.6. The labeled antibodies of the Singh disclosure are different from the conjugated antibody fragments of the present invention in three important respects.
 - (a) First, in the antibodies of Singh the cysteine residues of the interchain bond have not been replaced with another amino acid. Instead, the cysteine residues of the interchain bond are reduced at the same time as the cysteines in the hinge region, in order to create a thiol group that will accept a label.

- (b) Second, conjugating molecules to a Fab' fragment as in our invention presents different challenges than conjugating molecules to a whole antibody. A Fab' fragment, having no constant region extending beyond the hinge region, might be more subject to destabilizing effects of conjugated molecules; one skilled in the art might have expected the heavy and light chains to be more readily pulled apart if there were one or more molecules attached to them and there was no covalent bond between them. Thus, results obtained with conjugation of molecules on whole antibodies can not necessarily be used to predict the effect of conjugation of molecules to antibody fragments. In my opinion, the teachings of Singh relating to conjugated whole antibodies would not teach or suggest to one of ordinary skill in the art of antibodies and antibody fragments that similar results could be achieved with conjugated fragments having no covalent bond between the heavy and light chains and no constant region beyond the hinge. It is also significant that Singh does not teach the utility of said modified molecules *in vivo*.
- (c) Third, the labels used in Singh are very different from the effector molecules that we attached to the Fab' fragments. The label molecules of Singh were biotin-PEO-maleimide having a formula weight of 525.6Da (Singh, page 149, left column, lines 5-9). By contrast the effector molecules useful in the present invention are PEG or PEG derivatives having a molecular weight of about 5,000 – 40,000Da. In addition, hydrated PEG molecules were known to have an effective molecular size far in excess of 20kDa, typically in excess of 1 megaDa (Koumenis et al., *Int. J. Pharmaceutics* (2000) 198:83-95. Hence the potential for disruption or distortion of protein structure was perhaps significantly enhanced by the attachment of PEG rather than

biotin. In my opinion, one of ordinary skill in the art would appreciate that the results achieved by Singh using *small* molecules with *whole* antibodies could not be used to predict the results that would be achieved using *large* effector molecules with Fab' antibody *fragments*. Nor would the reference suggest to one skilled in the art to use either large effector molecules or Fab' fragments.

16. The Hsei reference teaches PEGylation of various antibody fragments, but does not teach that both interchain cysteines should be replaced with another amino acid. Hsei teaches nothing about modification of the interchain disulfide bond when the PEGylation takes place at the hinge, and provides no teaching or examples of antibodies or fragments in which the interchain cysteines on both the light chain and the heavy chain have been replaced. Hsei teaches that, at most, only one of the interchain cysteines is to be replaced with a serine, and that is to happen only when the other interchain cysteine is PEGylated (p. 23, lines 4-14; p. 24, line 24 – p. 27, line 7). Hsei provides no working example of an embodiment wherein one of the interchain cysteines is replaced and no working example of a Fab' fragment with more than one effector molecule. Hsei teaches nothing about modification of the interchain disulfide bond when the PEGylation takes place at the hinge. As one skilled in the art, this reference does not teach or suggest to me that it would be desirable to replace the cysteine residues in both the heavy and light chain of any antibody or fragment, and provides no motivation for doing so, nor does the reference teach or suggest that it would be desirable to add one or more effector molecules to such a modified fragment.
17. Hsei describes but does not exemplify the mutation of one or other of the interchain cysteines to serine in Fab, Fab' or F(ab')₂. Hsei describes but does not exemplify site specific attachment of a PEG molecule to any

cysteine other than a single cysteine in the hinge. Hsei describes but does not exemplify attachment of two PEG molecules to Fab or Fab'. Hsei describes but does not exemplify attachment of two PEG molecules to F(ab')₂ fragments using cysteine targeted PEG-maleimide reaction chemistry. Hsei describes and exemplifies attachment of 2 PEG molecules to F(ab')₂ molecules but only by using random amine (lysine) specific NHS-PEG chemistries followed by chromatographic separation of unPEGylated, monoPEGylated and diPEGylated F(ab')₂ fragments. Said F(ab')₂ molecules were formed from Fab' molecules with a C-terminal leucine zipper dimerisation motif followed by proteolytic cleavage and removal of the leucine zipper. Hsei neither describes nor exemplified methods for the reduction / activation of interchain cysteines for PEGylation without disruption of the inter-hinge disulphide bonds which stabilize the dimeric F(ab')₂ structure. Such a 'random PEGylation' approach was shown to be inefficient and wasteful by Hsei and had been evidenced in the prior art, (e.g. Pedley et al., Br. J. Cancer 1994 70:1126-1130, and Delgado et al., Br. J. Cancer 73:175-182). The approach taken by Hsei is clearly differentiated from the approach taken in our application which enabled specific control over both the site and number of PEG molecules attached. In addition our application describes precise methods for very efficient PEGylation reactions. Since Hsei did not make Fab' variants containing disrupted interchain disulphide bonds it was also not possible to foresee that one such variant forms very effectively a novel interchain disulphide bond between the C-terminal cKappa cysteine and the single hinge cysteine (see lane 4 figure 3b in our application), nor then that such a disulphide bond would retain very significant Fab' thermal stability (see pDPH225 in Table 1 of our application). Hsei does not teach any practicable or efficient method of PEGylating such molecules. We show in our application that even 5mM DTT compared to the 0.2mM DTT described in Hsei is ineffective at reducing any such 'unexpected' disulphide bonds to the extent required for high levels of PEGylation (see Figure 1 in our application). Furthermore, we

show in our application that stronger reducing conditions using different classes of reducing reagents (such as TCEP) are required in order to result in efficient PEGylation of such disulphide engineered Fab and Fab' molecules. It is also clear that 'strong' reducing reagents such as TCEP could not be used to reduce and PEGylate F(ab')₂ molecules without reducing them to Fab'.

18. As one of at least ordinary skill in the art, in my opinion there is no reason to combine either Hsei or Humphreys et al. (1997) with Singh et al. Singh et al. teaches whole antibodies, with all cysteines intact, and labeled with relatively small molecules. Hsei's teachings of various PEGylated fragments would teach nothing to the skilled artisan about Singh's whole antibodies. Humphreys (1997) teaches fragments that are neither labeled nor PEGylated, used to make F(ab')₂. One skilled in the art would see no reason to remove the cysteines from the interchain bonds as taught by Humphreys to make unlabelled F(ab')₂, in a process to make a labeled whole antibody or a fragment with an effector molecule, which fragment had to have at least one interchain cysteine intact. As one skilled in the art, I would have believed that the structure of Hsei with a broken interchain bond would have been unstable and have reduced binding affinity, and Hsei offered no stability data of such an embodiment to cause me to believe differently.

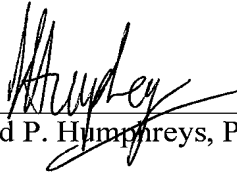
19. U.S 6,642,356 of which I am the sole inventor relates to peptides which function as hinge regions in antibodies and antibody fragments. This work is related to the work reported in my 1997 and 1998 papers, discussed above. Example 1 of this patent describes the production of di-Fab' from Fab' in E. coli. As stated in the patent (col. 9, lines 59-67), to minimize any possible incorrect interchain disulphide bonds between hinge regions and any other cysteines the interchain disulphide bond was removed from all Fab' constructs, by changing the interchain cysteines of cKappa and C_H1 to

serines. In particular, both cysteines were removed to minimize such incorrect bonds when the two Fab' fragments come together to form the di(Fab')₂ dimer. The issue of such incorrect bonds does not arise when the fragments are to be used in the form of un-dimerized Fab'. At no time in this example, or in this patent, is PEG or any other effector molecule attached to a Fab' fragment. The result of this example was that we discovered that the di-Fab' formation in vivo in the periplasm of E.coli is an inefficient process that is modulated, inter alia, by hinge sequence and complexity (col. 14, lines 33-35).

20. In Example 2 of the '356 patent I characterized two F(ab')₂ molecules with modified hinges. As reported at col. 18, line 28-52, one of the F(ab')₂ was PEGylated, with an efficiency of $\leq 1.3\%$, which we considered to be so low as to make this an unworkable process or method.
21. The SEQ ID NO:1 in the '356 patent is ^NTCPPCPXYCPPCPA^C, wherein X and Y which may be the same or different are each a neutral aliphatic L-amino acid residue. In one useful peptide X is an alanine residue and Y is a threonine residue. In the present application SEQ ID NO:1 is DKTHTCPP and SEQ ID NO:2 is DKHTTCAA. SEQ ID NO:1 of the '356 patent is not the same as SEQ ID NO:1 or SEQ ID NO:2 of the present application. In particular the sequences for the hinge region in the '356 patent contain 4 cysteine groups, while the hinge regions in the antibody fragments of the present application contain one or two cysteine groups. The additional cysteines in the hinge regions of the '356 patent fragments allow the Fab' fragments to dimerize with one another. In the present application the goal is not to dimerize the Fab' fragments.

22. I hereby state that I have been warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom, and I declare that all statements made of my own knowledge are true; and all statements made on information and belief are believed to be true.

Date: 17th August 2010



David P. Humphreys, PhD.

Publications

Humphreys DP and Bowering L (2009) Production of antibody Fab' fragments in *E. coli*, in Therapeutic Monoclonal Antibodies - from bench to clinic. Wiley and sons. p589-622.

Heywood SP and **Humphreys DP** (2009) Polymer fusions to increase antibody half-lives: PEGylation and other modifications, in Recombinant Antibodies for Immunotherapy. Cambridge University Press p275-292.

Humphreys D P, et al., (2007). Alternative antibody Fab' fragment PEGylation strategies: combination of strong reducing agents, disruption of the interchain disulphide bond and disulphide engineering. *PEDS* **20**: 227-234.

Humphreys D P (2007). Periplasmic expression of antibody fragments, in *The Periplasm*. ASM Press, Washington DC, pp361-388.

Humphreys D P (2004). Engineering of *Escherichia coli* to improve the purification of periplasmic Fab' fragments: changing the pI of the chromosomally encoded PhoS/PstS protein. *PROT. EXPR. PURIFIC.* **37**: 109-118.

Glover, D.J., and **Humphreys D P** (2004). Antibody fragments: production, purification and formatting for therapeutic applications, in *Antibodies Volume 1: production and purification*. Kluwer academic, London, pp. 25-73.

Humphreys D P (2003). Production of antibodies and antibody fragments in *Escherichia coli* and a comparison of their functions, uses and modification. *CURR. OPIN. DRUG DISC. DEV.* **6**: 188-196.

Humphreys D P et al., (2002). A plasmid system for optimization of Fab' production in *Escherichia coli*: importance of balance of heavy chain and light chain synthesis. *PROT. EXP. PURIFIC.* **26**: 309-320.

Humphreys D P, and Glover D J (2001). Therapeutic antibody production technologies: molecules, applications, expression and purification. *CURR. OPIN. DRUG DISC. DEV.* **4**: 172-185.

Humphreys D P, et al. (2000). High-level periplasmic expression in *E. coli* using a eukaryotic signal peptide: importance of codon usage at the 5' end of the coding sequence. *PROT. EXP. PURIFIC.* **20**: 252-264.

Humphreys D P, et al. (2000). Improved efficiency of site-specific copper(II) ion-catalysed protein cleavage effected by mutagenesis of cleavage site. *PROTEIN ENG.* **3**: 201-206.

Humphreys D P, et al. (1999). Efficient site specific removal of a C-terminal FLAG fusion from a Fab' using copper(II) ion catalysed protein cleavage. *PROTEIN ENG.* **12**: 179-184.

Humphreys D P, et al. (1998). F(ab')₂ molecules made from *E. coli* produced Fab' with hinge sequences conferring increased serum survival in an animal model. *J. IMMUNOL. METH.* **217**: 1-10.

Humphreys D P, et al. (1997). Formation of dimeric Fabs in *E. coli*: effect of hinge size and isotype, presence of interchain disulphide bond, Fab' expression levels, tail piece sequences and growth conditions. *J. IMMUNOL. METH.* **209**: 193-202.

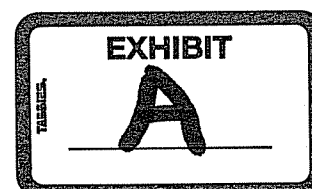
Humphreys D P, et al. (1996). Co-expression of human protein disulphide isomerase (PDI) can increase the yield of an antibody Fab' fragment expressed in *E. coli*. *FEBS LETTERS* **380**: 194-197.

Humphreys D P, et al. (1995). Human protein disulfide isomerase functionally complements a *dsbA* mutation and enhances the yield of pectate lyase C in *E. coli*. *J. BIOL. CHEM.* **270**: 28210-28215.

Stafford S J, **Humphreys D P**, and Lund P A (1999). Mutations in *dsbA* and *dsbB*, but not *dsbC*, lead to an enhanced sensitivity of *E. coli* to Hg²⁺ and Cd²⁺. *FEMS LETTERS.* **174**: 179-184.

Presentations: Oral

- "The cell as a manufacturing site" TOPRA Biotechnology, London 13th March 2008.
- "What is the potential for antibody fragments?" BioProduction, Berlin 30th October 2007.
- "Bacterial and yeast expression systems" TOPRA Biotechnology April 2007.



- "Antibody Fab' fragments: expression and purification from *E. coli*, PEGylation and clinical uses." Keystone, Lake Louise, 3rd Feb 2007.
- "Multi-PEGylation, an alternative and high efficiency strategy for the PEGylation of therapeutic antibody Fab fragments." HAH, Montego Bay, 12th May 2006.
- "PEGylation of antibody Fab' fragments: expression and purification from *E. coli*, PEGylation and clinical uses." World Biopharm Forum, Queens college Cambridge UK, 18th April 2006.
- "PEGylation of antibody Fab' fragments produced in *E. coli*." IBC Drug Disc. Tech., Hammersmith, London, 14th March 2006.
- "Production of antibodies and antibody fragments in microbial systems." Modern Drug Discovery & Development, San Diego, 19th October 2004.
- "Engineering of *E. coli* to improve the purification of periplasmic Fab' fragments: changing the pI of the chromosomally encoded PhoS/PstS protein". HAH, Dublin 8th October 2004.
- "CASE study: Simplifying the purification of periplasmic Fab', the creation an use of new *E. coli* strains". Visiongain Strategies for monoclonal antibody therapy 2004, London Kensington Hilton, 1-2 July 2004.
- "Engineering of *Escherichia coli* to improve the purification of periplasmic Fab' fragments: changing the pI of the chromosomally encoded PhoS / PstS protein". CHI Recombinant Antibodies conference, Cambridge MA, 28th April 2004.

Posters

"Multi-PEGylation, a new strategy for the high efficiency PEGylation of therapeutic antibody Fab' fragments" IBC Antibody Engineering, San Diego, 3-8th December 2005.

"Engineering of *Escherichia coli* to improve the purification of periplasmic Fab' fragments: changing the pI of the chromosomally encoded PhoS / PstS protein". IBC Antibody Engineering, San Diego 1-4 December 2003.

"High yielding and cost effective production of humanised Fab' fragments in *E. coli*". IBC Antibody Engineering, San Diego 3-6 December 2000.

"High yielding and cost effective production of functionally different humanised antibody fragments in *E. coli*". Medicon Valley Association, Malmö, Sweden 20-21 September 2000.

"High yielding and cost effective production of functionally different Humanised Antibody fragments in *E. coli*". Human Antibodies and Hybridomas, Edinburgh, 8-10 Sept. 1999.

"F(ab')₂ molecules with hinge sequences conferring increased resistance to chemical reduction *in vitro* and increased serum permanence times in an animal model". Therapeutic Antibodies, San Francisco 21-24 September 1997

"Co-expression of Human Protein Disulphide Isomerase (PDI) can increase the yield of an antibody Fab' fragment expressed in *Escherichia coli*". NIH conference on Protein Folding, Washington DC, 23-26 April 1996.

Patent applications

WO2005/003169 Fab PEGylation construct.
 WO2005/003170 MultiPEGylation.
 WO2005/003171 Disulphide engineering / PEGylation.
 WO2004/035792A1: PhoS strain engineering.
 WO2003/004636A2: Bacteriophage signal peptides.
 WO0032795A1: Copper cleavage peptides.
 WO9915549A2 / US6642356B1 / EP1015495B1: Hinge sequences.
 GB 95/18383.6, "Microbiological process".

